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Development of Tethered Hsp90 Inhibitors Carrying Radioiodinated Probes To Specifically Discriminate and Kill Malignant Breast Tumor Cells

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14. ABSTRACT
We have proposed to develop tethered Hsp90 inhibitor capable of carrying various radioactive iodine isotopes for early detection and ablation of metastatic breast cancers. These probes specifically target a surface form of heat shock protein 90 (eHsp90) that is expressed on malignant cells and internalized. In this report we describe our chemistry efforts to synthesize a non-radioactive tethered Hsp90 inhibitor and methods developed for stannylation of the molecule such that it can be effectively radioiodinated with either ^{131}I or ^{124}I. Two paths of synthesis were developed and optimized. A standard operating procedure was developed and then adapted and modified to provide workable protocols for radioiodination. An inactive control molecule was also developed, which can also be labeled with radioiodine to permit determination of specificity of radio-ligand localization. These molecules will be tested as imaging agents in mouse models of breast cancer.

15. SUBJECT TERMS
Radiodination, tethered Hsp90 inhibitor, malignant breast tumor, ectopic Hsp90

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1. Introduction

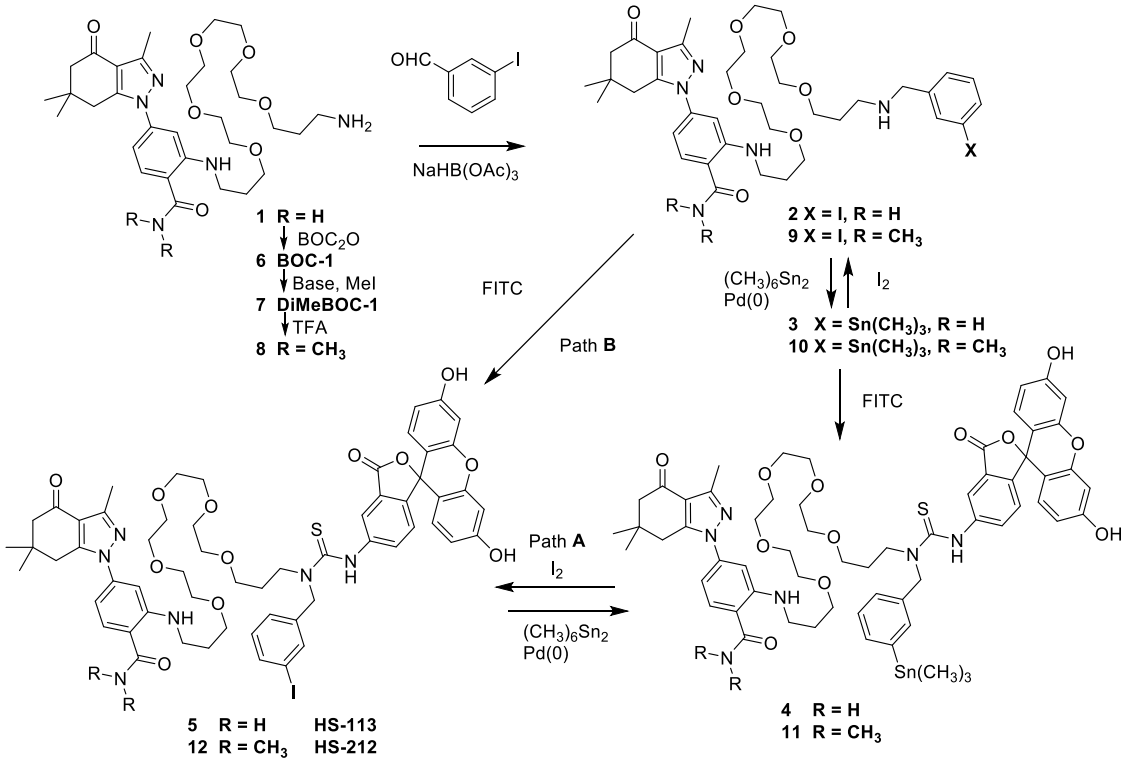
In the US, routine breast cancer screening results in over 1.6 million biopsies annually leading to the diagnosis and surgical resection of breast cancer or breast carcinoma *in situ* in over 250,000 women respectively. Unfortunately, the sensitivity but low specificity of screening has led to concerns about over treatment of indolent disease, as evidenced by the increased incidence and treatment of early stage breast cancer without a concomitant decrease in the nearly 40,000 breast cancer deaths annually. Clinical data indicate a strong link between high expression/activation of Heat shock protein 90 (Hsp90) with poor prognosis in malignant breast cancer (1, 2). Specifically, immunohistochemical analysis of breast cancer cell lines and 655 primary breast cancers (including 331 ER+ and 324 ER- tumors) showed increased Hsp90 expression in all breast cancer cell lines, and in nearly 90% of primary breast cancers (2). A recent study at our institution evaluated Hsp90 gene expression from profiles of over 4,000 breast cancer patients from 23 publically available gene expression databases, which also reported overall survival data from over 1000 patients. This study confirmed up regulated Hsp90 was associated with poor overall survival in all breast cancer subtypes including estrogen (ER) negative, HER2 negative and triple negative breast cancers (1). Our laboratories recently developed a series of optical and iodinated tethered Hsp90 inhibitors that have exquisite selectivity *in vivo* for metastatic breast tumors expressing ectopic (cell surface) Hsp90 (3). We also discovered that ectopically expressed Hsp90 is rapidly internalized and can carry these tethered inhibitors specifically into the breast cancer cells. This work in tandem with published clinical results suggests that selective targeting of Hsp90 up regulated in malignancy may present an opportunity to not only discriminate indolent tumors from metastatic disease, but also offer a molecularly targeted radiotherapy approach for body wide tumor ablation with low normal tissue toxicity. Herein, we propose to develop a series of tethered Hsp90 inhibitors capable of selectively delivering radioiodine (^{124}I and ^{131}I) or ^{211}At to malignant tumor cells. We envisage a process in which a patient, after standard of care breast exam, is first evaluated for malignancy vs. indolent disease by positron emission tomography (PET) imaging using ^{124}I-labeled tethered inhibitors. Then, in patients with malignancies detected in high contrast to normal tissues, targeted radiotherapy would be preformed at patient-optimized doses of inhibitor labeled with the β-emitter ^{131}I or the α-emitter ^{211}At. **This is an attractive strategy for breast cancer because the same molecules can be used to not only discriminate indolent disease from metastatic, but also enables selective tumor ablation on a personalized level, potentially mitigating life altering side effects commonly associated with current chemotherapeutics or radiation strategies**.

2. Keywords

Radioiodinated, Tethered Hsp90 inhibitor, malignant breast cancer, stannylation.

3. Accomplishments.

As mentioned above, we previously reported both fluorescein and iodinated probes(*1*). To get the cellular selectivity of our fluorescein probe along with the capabilities of carrying a putative, PET/SPECT imaging agent or radio toxic agent, we need a probe with both imaging moieties. Such a synthesis requires compound that can be easily be converted to the desired radio-labeled iodine compound at a later stage. The approach, as shown in Scheme **1**, allows for introduction of a variety of iodine isotopes, via tin-iodine exchange, at two alternative steps in the procedure. Thus (path **A**), compound **1** is converted, via reductive amination to the aryl iodide **2**. Compound **2** is converted by palladium mediated halogen-tin exchange(*2*) to the tin derivative **3**. This compound is further treated with FITC (fluorescein isothiocyanate) to give the precursor **4**, which can be converted with hot iodine(*3*) to the desired product **5**. Conversely (path **B**), compound **3** can be converted back to labeled **2**, which can then be reacted with FITC to give the final product **5**. Both routes have been explored successfully with cold iodine. The iodination step from the tin precursor was much cleaner when the tin was sequestered in the reaction. Reaction paths **A** and **B** have been successfully explored with ^{124}I. It is also possible to convert compound **5** to compound **4** using the palladium mediated halogen-tin exchange reaction.



Scheme **1**. Two paths to the synthesis a probe incorporating both fluorescein and hot iodine for both active (**5**) and inactive (**12**) probes.

To obtain a control compound with very similar physical properties but lacking the ability to bind to Hsp90, we also made a dimethyl amide analog **12**. The addition of the methyl groups blocks an important hydrogen bond between the Hsp90 ligand and an aspartic acid on Hsp90 in the active site. Control analog **12** was made in an analogous fashion to compound **5**, as shown in Scheme **1**. The control chemistry has not been explored with ^{124}I. Compounds **5** and **12** are referred to internally as **HS-113** and **HS-212**, respectively. Experimental details are included in the appendix.

(1) Barrott, J. J., Hughes, P. F., Osada, T., Yang, X. Y., Hartman, Z. C., Loiselle, D. R., Spector, N. L., Neckers, L., Rajaram, N., Hu, F. Y., Ramanujam, N., Vaidyanathan, G., Zalutsky, M. R., Lyerly, H. K., and Haystead, T. A. (2013) Optical and Radioiodinated Tethered Hsp90 Inhibitors Reveal Selective Internalization of Ectopic Hsp90 in Malignant Breast Tumor Cells. *Chemistry & Biology 20*, 1187-1197.

(2) Chun, J.-H., and Pike, V. W. (2012) Regiospecific Syntheses of Functionalized Diaryliodonium Tosylates via Hydroxy(tosyloxy)iodo arenes Generated in Situ from (Diacetoxyiodo)arenes. *Journal of Organic Chemistry 77*, 1931-1938.

(3) Koziorowski, J., Henssen, C., and Weinreich, R. (1998) A new convenient route to radioiodinated N-succinimidyl 3- and 4-iodobenzoate, two reagents for radioiodination of proteins. *Applied Radiation and Isotopes 49*, 955-959.

4. Impact. The successful synthesis of both and active and inactive tethered Hsp90 carrying iodine that can be successfully radioiodinated is an important milestone. Two methods of synthesis were developed that will greatly facilitate translation of this technology to clinical practice. The Zalutsky laboratory have also developed methodologies that enable the radioiodination of both I121 and I 124 versions reproducibly. Work is currently ongoing to radio-iodinate the inactive control molecule. This latter molecule will validate that our probes are specifically targeting tumors by binding surface Hsp90. Next steps will involve testing these newly synthesized molecules in mouse models of breast cancer. Success in these experiments will enable us to advance the project to testing the effects of delivery lethal forms of iodine such I131 or the alpha emitter At211.

5. Changes/Problems.

None to report

6. Products

Two novel radio-iodinated tethered Hsp90 inhibitors have been developed shown in Scheme **1**.

7. Participants & Other Collaborating Organizations……

Grants.gov ID Number	W81XWH-15-1-0073
Principal Investigator	Timothy Haystead
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Contracting Organization	Duke University
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Indirect Costs	$199,500

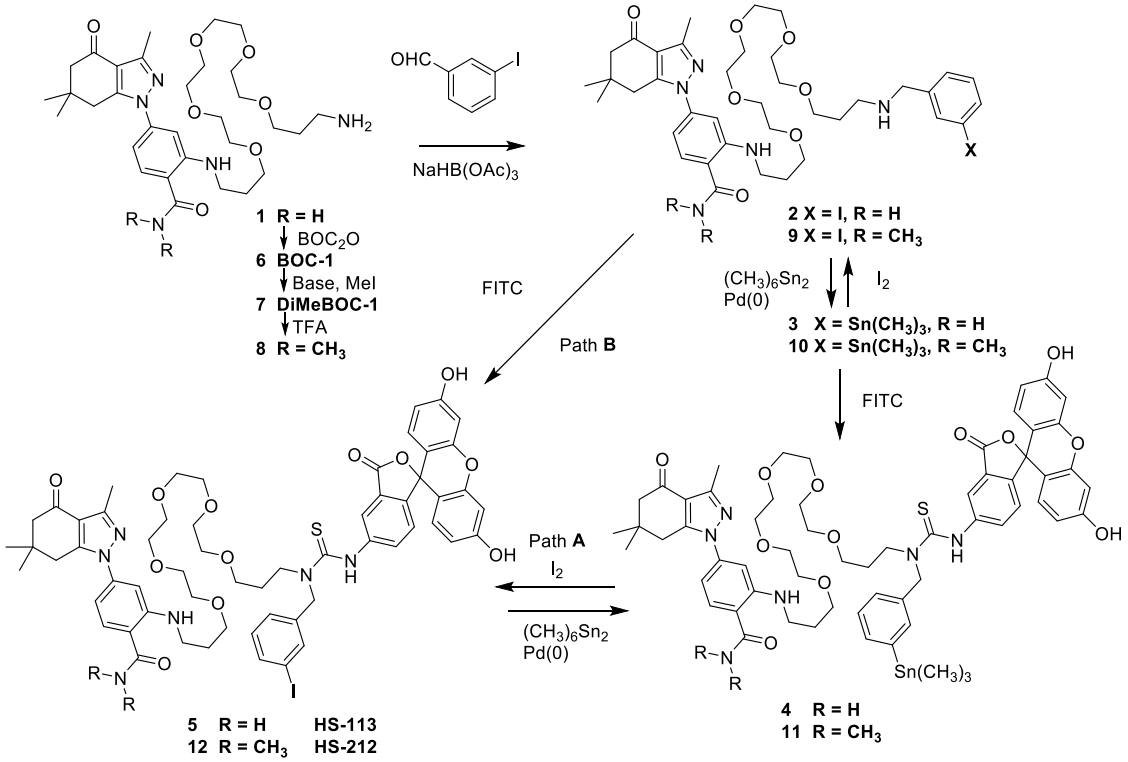
8. Special Reporting Requirements

none

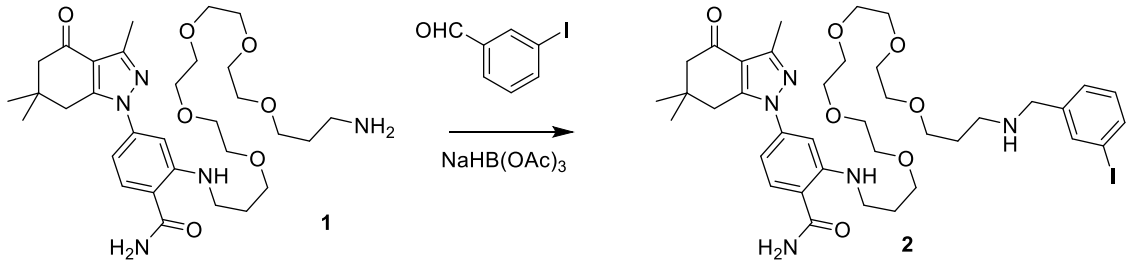
9. Appendices

Synthetic experimental details

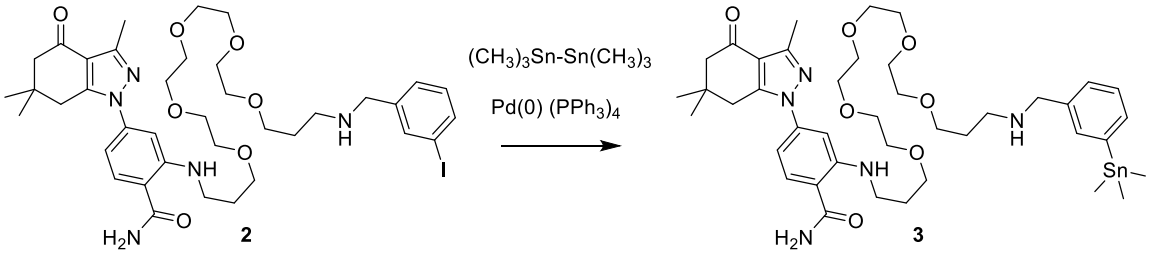
Reagents were obtained from commercial sources and used without further purification. Proton NMR spectra were obtained on Varian 400 and 500 MHz spectrometers. LC/MS were obtained on an Agilent ion-trap LC/MS system. HRMS results were obtained on an Agilent 6224 LCMS-TOF and are reported as an average of four runs. Compound **1** was previously reported(*1*).



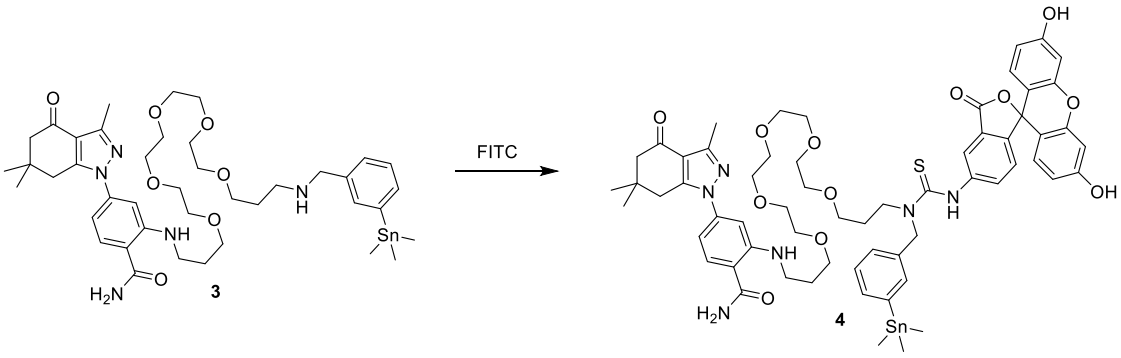
Synthetic scheme for compounds described below.



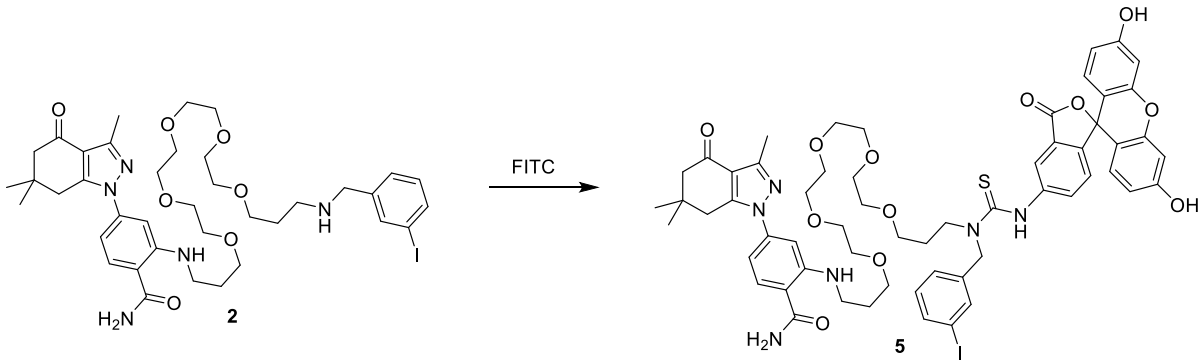
Amine **1** (260 mg, 431 μmol) and 3-iodobenzaldehyde (105 mg, 452 μmol) were dissolved in dichloroethane (4 mL) and treated with sodium triacetoxyborohydride (137 mg, 646 μmol) and stirred at RT for 3 days. The reaction mixture was then concentrated and chromatographed (silica gel, 0 to 20% 9/1 : MeOH/NH_3 in CH_2Cl_2) to give iodide **2** (203 mg, 57%) as a clear oil. LC/MS gives m/z = 820.4 $[M+1]^+$. Bisalkylation product was also isolated (91 mg, 21%) as an oil. LC/MS gives m/z = 1036.3 $[M+1]^+$. ^{1}H-NMR (DMSO-d_6) δ 8.41 (t, J = 5 Hz, 1H), 7.92 (br s, 1H), 7.74 (d, J = 8 Hz, 1H), 7.76 (br s, 1H), 7.56 (d, J = 8 Hz, 1H), 7.32 (d, J = 8 Hz, 1H), 7.26 (br s, 1H), 7.10 (t, J = 8 Hz, 1H), 6.77 (d, J = 2 Hz, 1H), 6.67 (dd, J = 2, 8 Hz, 1H), 3.63 (s, 2H), 3.38-3.52 (m, 20H), 3.20 (q, J = 6 Hz, 2H), 2.92 (s, 2H), 2.40 (s, 3H), 2.32 (s, 2H), 1.80 (p, J = 6 Hz, 2H), 1.62, (p, J = 6 Hz, 2H), 1.01 (s, 6H).



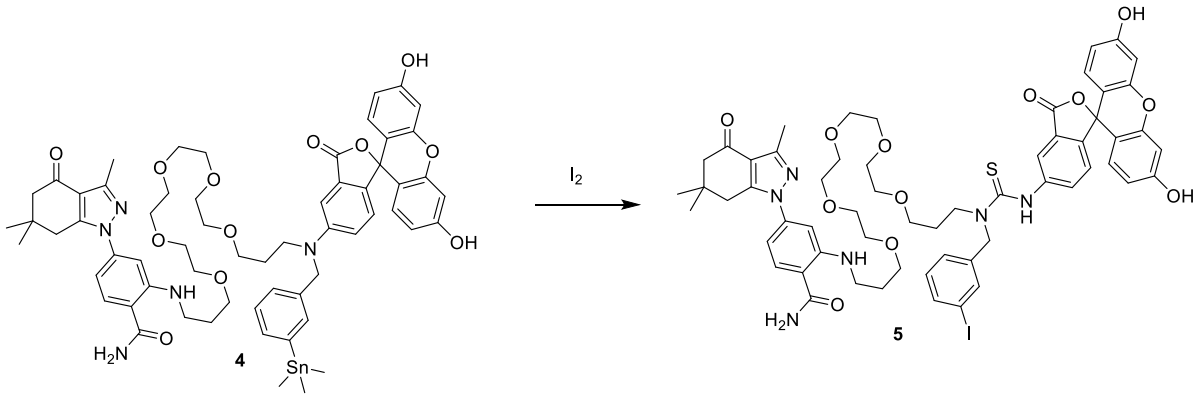
Aryliodide **2** (230 mg, 280 μmol), hexamethylditin (101 mg, 308 μmol) and tetrakis triphenylphosphine palladium(0) (6.48 mg 5.61 μmol) were slurried in dioxane (5 mL), purged with nitrogen and heated to 100 ºC for 45 m. The reaction mixture was then concentrated and chromatographed (silica gel, 0 to 10% 9/1 : MeOH/NH_3 in CH_2Cl_2) to give the tin compound **3** (213 mg, 89%) as a glass containing dark material (Pd?). The sample was further purified by prep HPLC (Agilent Prep C-18, 2.5 x 25 cm, 30 to 100 % MeOH w/2% formic acid, 20 mL/min) and concentrated to give the tin product **3** (78 mg) as a clear oil. LC/MS gave a single peak with m/z = 858.4 $[M+1]^+$. ^{1}H NMR ($CDCl_3$) δ 8.35 (s, 1H, formate), 7.99 (br s, 1H), 7.53 (d, J = 8.4 Hz, 1H), 7.49 (br s, 1H), 7.43 (dm, J = 7 Hz, 1H), 7.34 (dm, J = 7 Hz, 1H), 7.29 (dd, J = 7 Hz, 1H), 6.74 (d, J = 2 Hz, 1H), 6.55 (dd, J = 2, 8.4 Hz,1H), 6.44 (v br s, 1H), 4.06 (s, 2H), 3.49-3.59 (m, 16H), 3.47 (m, 4H), 3.24 (t, J = 6.2 Hz, 2H) 3.06 (t, J = 6.2 Hz, 2H), 2.77 (s, 2H), 2.48 (s, 3H), 2.34 (s, 2H), 1.93 (p, J = 6.2 Hz, 2H), 1.88 (p, J = 6.2 Hz, 2H), 1.05 (s, 6H), 0.23 (s et al, 9H); ^{13}C NMR ($CDCl_3$) δ 193.7, 171.79, 167.09 (formate), 151.14, 149.98, 149.31, 143.81, 142.68, 137.35, 136.77, 131.11, 130.01, 129.69, 128.65, 117.26, 112.86, 109.06, 106.33, 70.67, 70.63, 70.55, 70.51, 70.47, 70.38, 70, 69.64, 69.08, 52.52, 50.92, 45.88, 41.01, 40.34, 37.66, 35.97, 29.13, 28.54, 25.45, 13.56, -9.36.



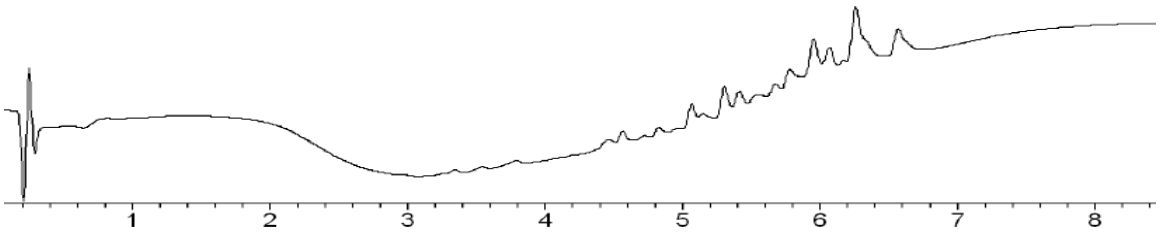
Aryltin compound **3** (116 mg, 135 μmol) was dissolved in DMSO (200 μL) and treated with FITC (53 mg, 135 μmol) dissolved in DMSO (200 μL) followed by Hunig's base (around 35 mg, 270 μmol). The mixture was purified by prep HPLC (30 to 100% methanol, 20 mL/m, Agilent C-18, 21.1 x 25 cm) to give product **4** (68 mg, 40%) as a yellow solid. LC/MS gave m/z = 1247.4 $[M+1]^+$. ^{1}H-NMR (DMSO-d_6) δ 10.17 (br s, 2H), 9.46 (br s, 1H), 8.40 (br t, 1H), 7.94 (s, 1H), 7.92 (br s, 1H), 7.74 (2d, 2H), 7.42 (s, 1H), 7.39 (d, 1H), 7.33 (t, 1H), 7.25 (br s, 1H), 7.23 (d, 1H), 7.18 (d, 1H), 6.76 (s, 1H), 6.67 (br m, 3H), 6.57 (br s, 4H), 5.14 (s, 2H), 3.78 (m, 2H), 3.41-3.51 (m, 20H), 3.20 (m, 2H), 2.91 (s, 2H), 2.39 (s, 3H), 2.32 (s, 2H), 1.89 (m, 2H) 1.80 (m, 2H), 1.00 (s, 6H), 0.25 (s, 9H).



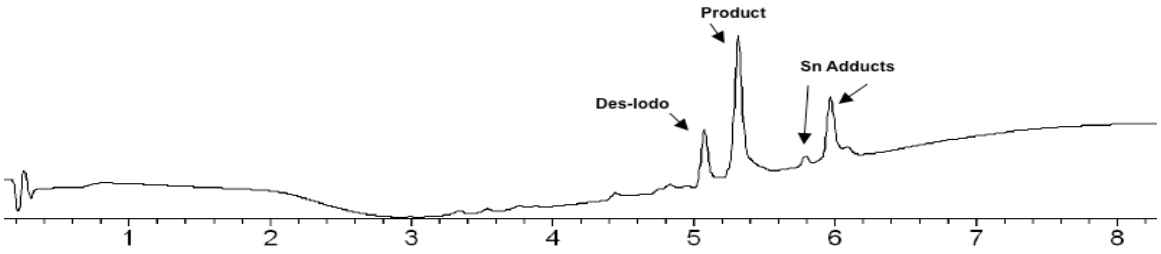
HS-113. Aryliodide **2** (31 mg, 38 μmol) was dissolved in DMSO (200 μL) and treated with FITC (15 mg, 38 μmol) dissolved in DMSO (200 μL) and Hunig's base (10 mg, 76 μmol). After an hour the compound was chromatographed (silica gel, 0 to 10% 10/1 : MeOH/AcOH in CH_2Cl_2) to give iodide **5** (28 mg, 62%) as a yellow glass. LC/MS gave m/z = 1207.4 $[M+1]^+$. ^{1}H-NMR (DMSO-d_6) δ 10.13 (br s, 2H), 9.47 (br s, 1H), 8.40 (br t, 1H), 7.95 (s, 1H), 7.92 (br s, 1H), 7.77 (d, J = 8 Hz, 1H), 7.73 (d, J = 8 Hz, 1H), 7.69 (s, 1H), 7.64 (d, J = 8 Hz, 1H), 7.33 (d, J = 8 Hz, 1H), 7.25 (br s, 1H), 7.15-1.20 (m, 2H), 6.76 (s, 1H), 6.67 (br m, 3H), 6.57 (br s, 4H), 5.14 (s, 2H), 3.74 (m, 2H), 3.38-3.51 (m, 20H), 3.19 (m, 2H), 2.91 (s, 2H), 2.39 (s, 3H), 2.31 (s, 2H), 1.87 (m, 2H) 1.79 (m, 2H), 1.00 (s, 6H).



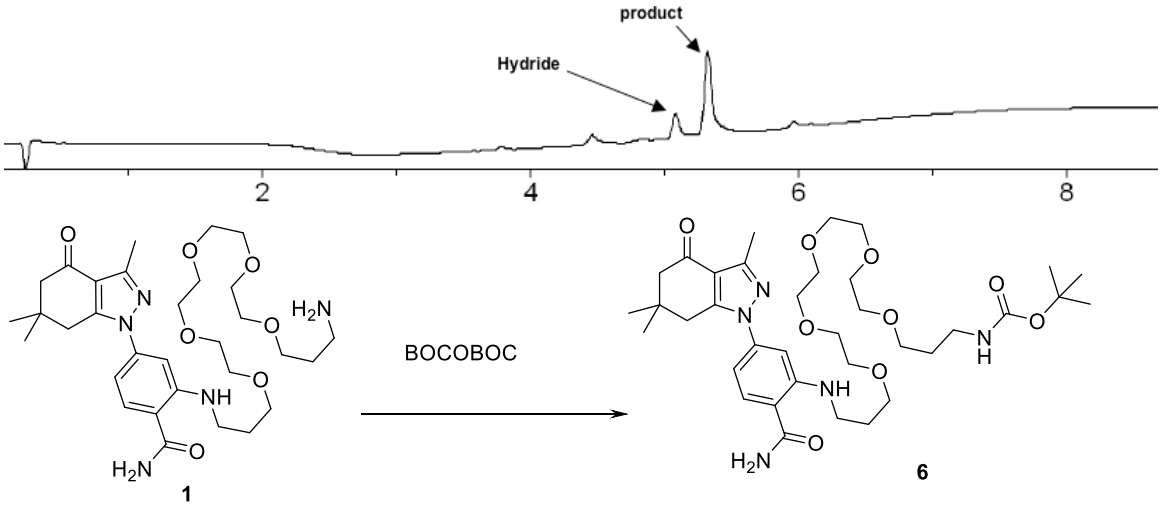
HS-113. Tin compound **4** (1 mg, 0.8 μmol) and sodium acetate (3 mg) were dissolved in methanol (1 mL) and treated iodine (8 μL of 0.1 M solution). After about 20 m, an aliquot was removed and analyzed by LC/MS which showed multiple products.



Tin compound **4** (1 mg, 0.8 μmol) was dissolved in methanol (1 mL) and treated with 1N HCl (3 drops) followed by iodine (8 uL of 0.1 M solution). After about 20 m, an aliquot was removed and analyzed by LC/MS which showed cleaner reaction but with some tin adducts.

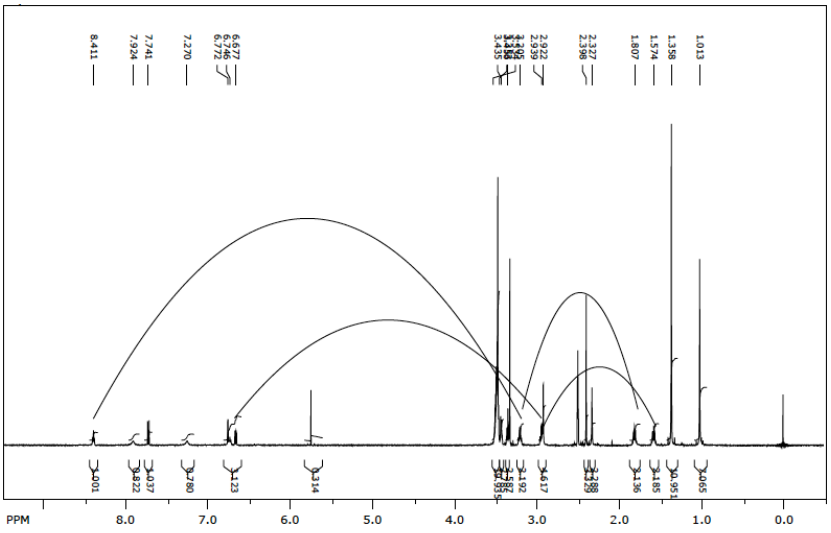


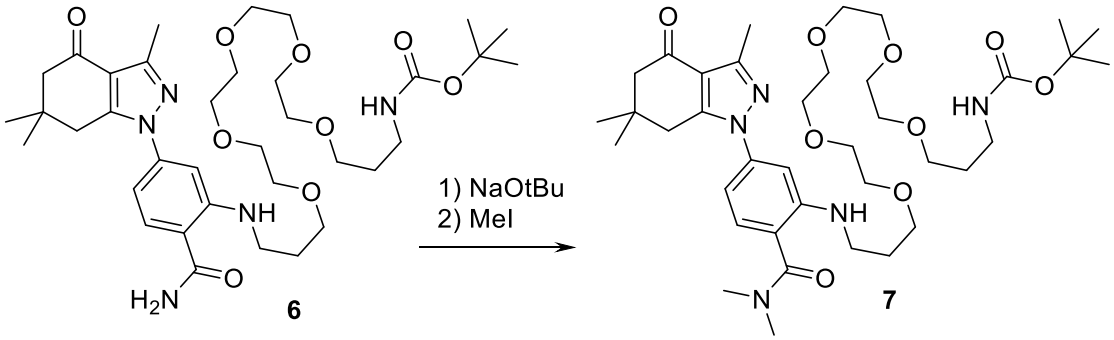
Tin compound **4** (1 mg, 0.8 μmol) was dissolved in methanol (1 mL) and treated with ethylenediamine di-HCl (1 mg in 10 μL of water) followed by iodine (8 μL of 0.1 M solution). The color dissipated instantly. The next day, an aliquot was removed and analyzed by LC/MS which showed the cleanest formation of product **5**.



Amine **1** was dissolved in methylene chloride (50 mL) and treated with di-tert-butyl dicarbonate (940 mg, 1.04 mL, 4.5 mmol). After 30 minutes the reaction mixture was concentrated and chromatographed (silica gel, 0 to 2.5% MeOH in CH_2Cl_2) to give the product **6** (1.52 g, 50%) as a clear viscous oil. LC/MS gave a single peak with m/z = 704.4, ^{1}H NMR (DMSO-d_6) δ 8.41 (br t, J = 6 Hz, 1H), 7.92 (br s, 1H), 7.74 (d, J = 8 Hz, 1H), 7.27 (br s, 1 H) 6.77 (d, J = 2 Hz, 1H), 6.75 (br t, J = 6 Hz, 1H), 6.68 (dd, J = 2, 8 Hz, 1H), 3.46-5.53 (m, 18H), 3.44 (m, 2H), 3.35 (t, J = 6 Hz, 2H), 3.2 (q, J = 6 Hz, 2H), 2.94 (q, J = 6 Hz, 2H), 2.92 (s, 2H), 2.40 (s, 3H), 2.33 (s, 2H), 1.81 (p, J = 6 Hz, 2H), 1.57 (p, J = 6 Hz, 2H), 1.36 (s, 9H), 1.01 (s, 6H).

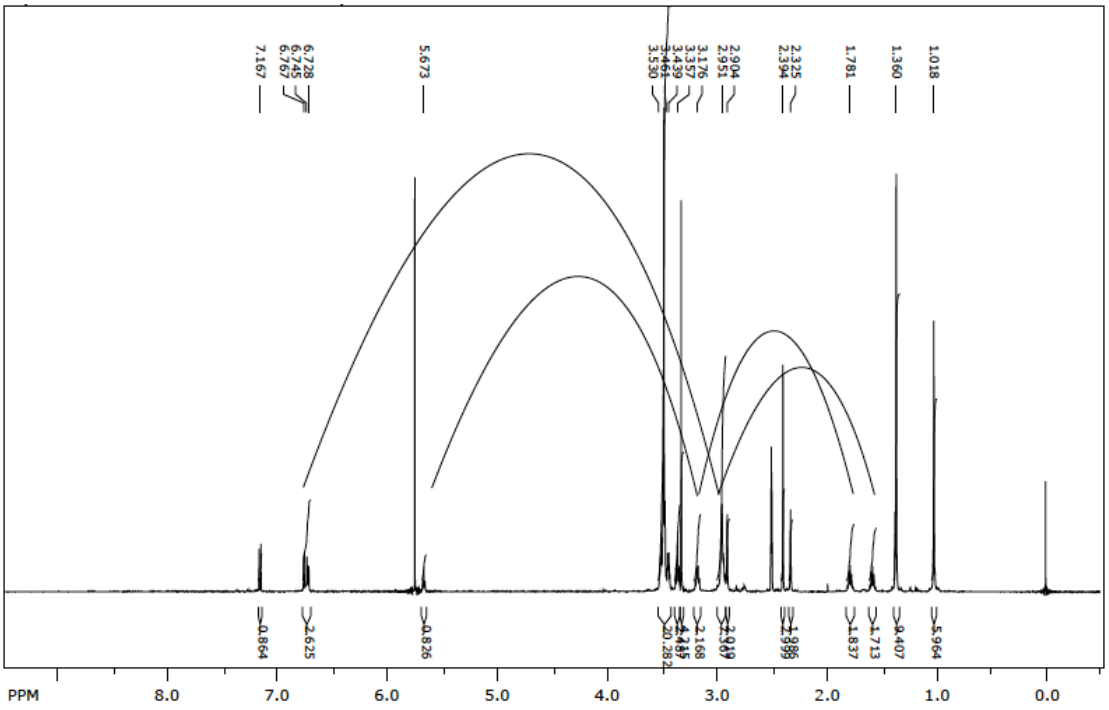
Arcs show coupling seen in COSY-NMR.

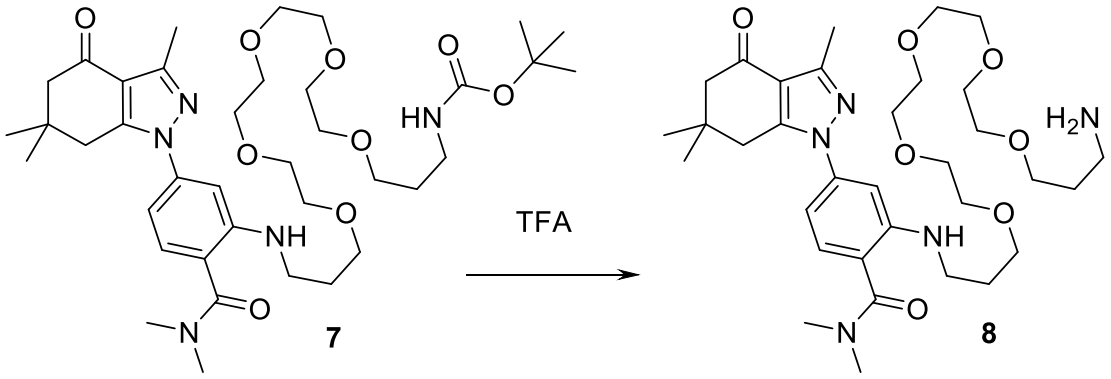




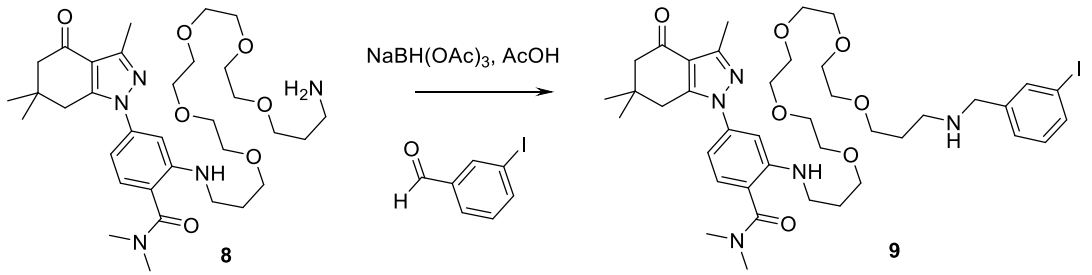
BOC amide **6** (893 mg, 1.27 mmol) was dissolved in THF (20 mL) and treated with potassium t-butoxide (142 mg, 1.27 mL of 1N solution in THF, 1.27 mmol) followed by slow addition of methyl iodide (180 mg, 79 μL, 1.27 mmol).and stirred at RT. TLC (9/1 : CH_2Cl_2 /MeOH and EtOAc/MeOH) after about 1h shows fairly clean formation of mono and di alkylation plus starting material. The reaction was again treated with base and iodide (same amounts). TLC after 30 m shows the same only tilted more toward di. The entire reaction mixture was added to silica gel (7 g) and concentrated and chromatographed (silica, 0 to 30% MeOH in EtOAc) to give **7** (397 mg, 43%) as a viscous yellow oil. LC/MS gave a single peak (>95%) with m/z = 732.5. ^{1}H NMR (DMSO-d_6) δ 7.17 (d, J = 8 Hz, 1H), 6.77 (d, J = 2 Hz, 1H), 6.75 (br t, 1H), 6.73 (dd, J = 2, 8 Hz, 1H)), 5.67 (br t, J = 6 Hz,1H), 3.46-3.53 (m, 18H), 3.44 (m, 2H), 3.36 (t, J = 6 Hz, 2H), 3.18 (q, J = 6 Hz, 2H), 2.95 (br s, 6H), (2.94 (q, J = 6 Hz, 2H), 2.90 (s, 2H), 2.39 (s, 3H), 2.33 (s, 2H), 1.78 (p, J = 6 Hz, 2H), 1.58 (p, J = 6 Hz, 2H), 1.36 (s, 9H), 1.02 (s, 6H).

Arcs show coupling in COSY-NMR. Only the non-coupled exchangeable protons go away on di-methylation.



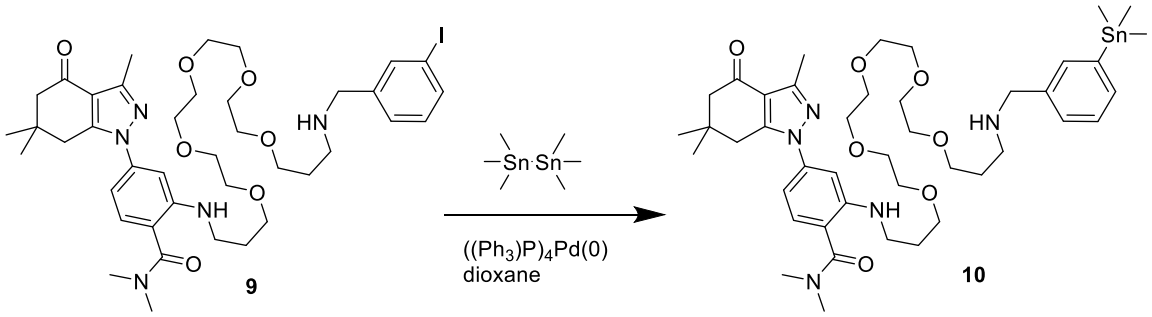


The dimethyl BOC compound **7** (338 mg, 0.46 mmol) was treated with TFA in CH_2Cl_2 for 1 h. This sample was concentrated and purified by prep LC (0 to 100% methanol, 20 mL/m, Agilent C-18, 21.1 x 25 cm) to give **8** (137 mg, 47%) as a clear oil. LC/MS gave a single peak with m/z = 632. 4 $[M + 1]^+$. ^{1}H-NMR (DMSO-d_6) δ 7.61 (br s, 2H), 7.17 (d, J = 8 Hz, 1H), 6.77 (d, J = 2 Hz, 1H), 6.73 (dd, J = 2, 8 Hz), 5.67 (br s, 1 H), 3.43-3.54 (m 20 H), 3.18 (m, 2H), 2.95 (br s, 6H), 2.90 (s, 2H), 2.85 (m, 2H), 2.40 (s, 3H), 2.33 (s, 2H), 1.72-1.81 (m, 4H), 1.02 (s, 6H).

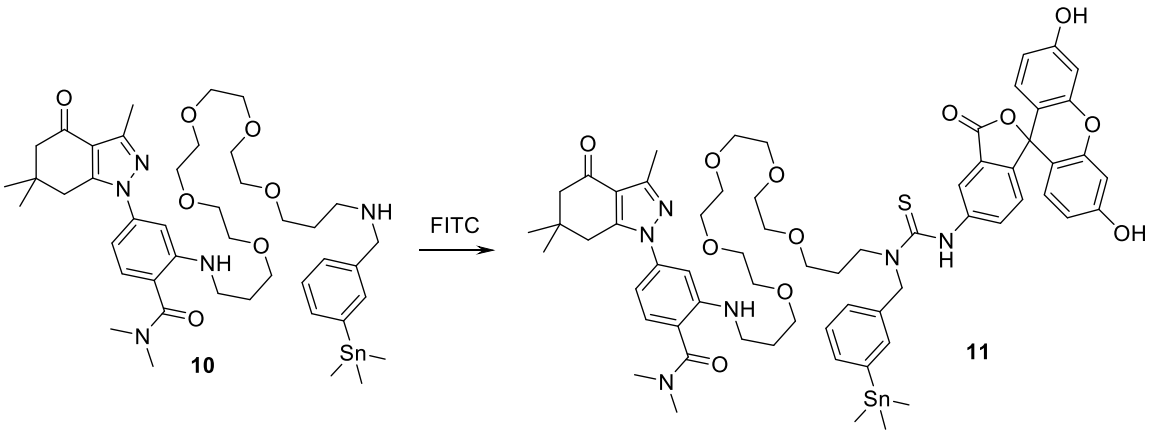


Amine **8** (133 mg, 210 µmol) was dissolved in methylene chloride (2 mL) and treated sequentially with 3-iodobenzaldehyde (54 mg, 231 µmol), acetic acid (24 µL), and sodium

triacetoxyborohyudride (112 mg, 526 μmol) and stirred at RT. After 1 h, more sodium triacetoxyborohyudride (112 mg, 526 μmol) was added. An hour later the reaction mixture was added to silica, concentrated and chromatographed (silica gel, 0 to 20% 9/1 : MeOH/NH_4OH in CH_2Cl_2) to give the monoalkylated product **9** (69 mg, 38%) as a clear glass. Bis-alkylated product (21 mg) was also isolated as a clear glass. LC/MS gave a peak with m/z = 1063.9 $[M+1]^+$. ^{1}H-NMR (DMSO-d_6) δ 7.86 (s, 1H), 7.74 (d, J = 7.5 Hz, 1H), 7.45 (d, J = 7.5 Hz, 1H), 7.21 (t, J = 7.5 Hz, 1H), 7.17 (d, J = 8 Hz, 1H), 6.77 (d, J = 2 Hz, 1H), 6.73 (dd, J = 2, 8 Hz), 5.67 (br s, 1 H), 4.01 (br s, 2H), 3.42-3.54 (m 20 H), 3.17 (m, 2H), 2.95 (br s, 6H), 2.90 (s, 2H), 2.87 (m, 2H), 2.39 (s, 3H), 2.32 (s, 2H), 1.74-1.83 (m, 4H), 1.02 (s, 6H).

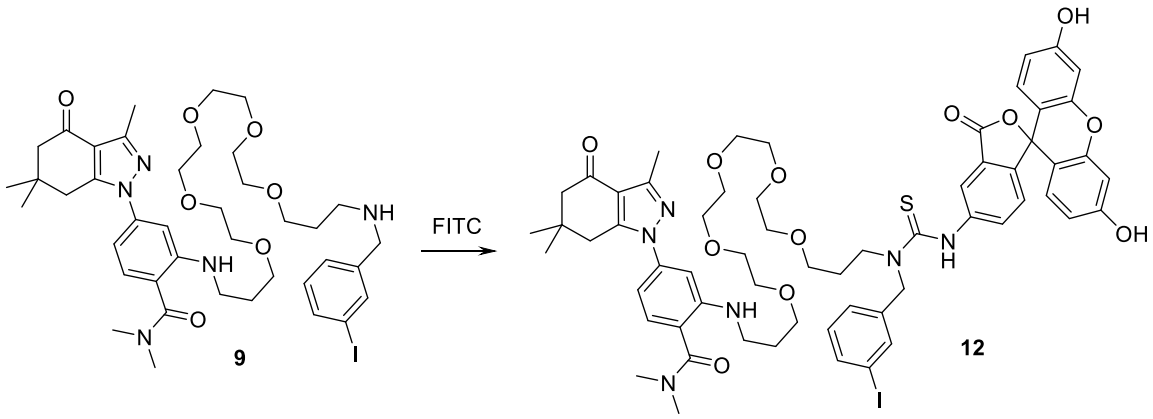


Iodide **9** (88 mg, 104 μmol), hexamethylditin (44 mg, 28 μL, 135 μmol) and tetrakis triphenylphosphine palladium(0) (2.4 mg 2.1 μmol) were slurried in dioxane (2 mL), purged with nitrogen for 30 m and heated to 100 ºC for an hour. TLC (9/0.9/0.1 : CH_2Cl_2/MeOH/NH_4OH) showed complete conversion to product. The reaction mixture was concentrated and chromatographed (silica gel, 0 to 25% 9/1 : MeOH/NH_4OH in CH_2Cl_2) to give product tin product **10** (78 mg, 83%) as a glass. LC/MS gives m/z = 886.4 $[M+1]^+$. ^{1}H-NMR (DMSO-d_6) δ 8.42 (v br s, 1H), 7.58 (br s, 1H), 7.51 (br m, 1H), 7.39 (br m, 2H), 7.17 (d, J = 8 Hz, 1H), 6.77 (br s, 1H), 6.73 (br d, J = 8 Hz, 1H), 5.67 (t, J = 5 Hz, 1H), 4.10 (br s, 2H), 3.43-3.52 (br m, 20H), 3.17 (q, J = 6 Hz, 2H), 2.97 (m, 2H), 2.97 (br s, 6H), 2.90 (s, 2H), 2.39 (s, 3H), 2.33 (s, 2H), 1.83 (m, 2H), 1.78 (m, 2H), 1.02 (s, 6H), 0.28 (s, 9H).

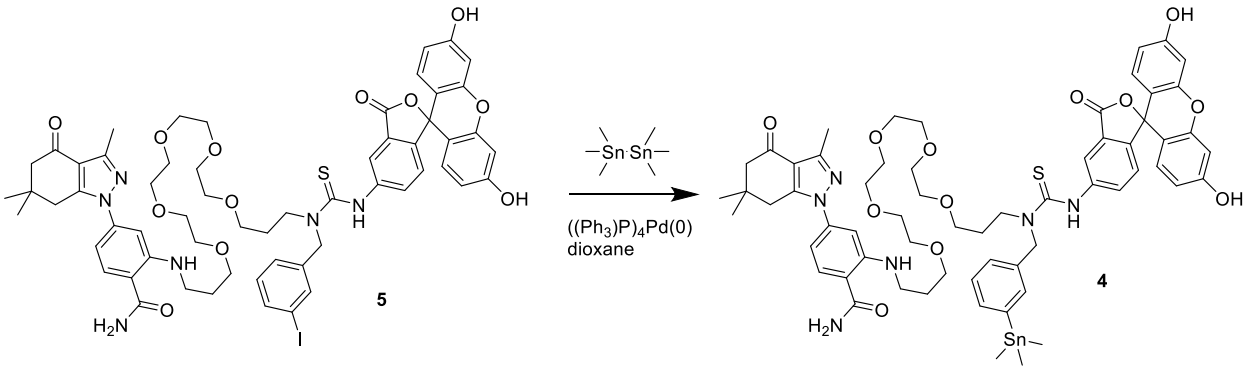


Tin compound **10** (40 mg, 45 μmol) was dissolved in ethanol (2 mL) and treated with Hunig's base (2 drops) followed by FITC (21 mg, 54 μmol). After 30 m, TLC (4/0.9/0.1 : CH_2Cl_2/MeOH/NH_4OH & 9/1 : CH_2Cl_2/MeOH) showed no starting amine and a new product. The reaction mixture was concentrated and chromatographed (silica gel, 0 to 20% MeOH in CH_2Cl_2) to give tin adduct **11** (33 mg, 57%) as a yellow glass. LC/MS gave a major peak with

m/z = 1275.5 $[M+1]^{+1}$. $]^{+}$. ^{1}H-NMR (DMSO-d_6) δ 10.15 (br s, 2H), 9.45 (br s, 1H), 7.94 (s, 1H), 7.74 (d, J = 8 Hz, 1H), 7.43 (s, 1H), 7.38 (m, 1H), 7.33 (m, 2H), 7.24 (d, J = 8 Hz, 1H), 7.17 (t, J = 8 Hz, 1H) 7.16 (d, J = 8 Hz, 1H), 6.76 (s, 1H), 6.72 (d, J = 8 Hz, 1H), 6.67 (br m, 2H), 6.57 (br m, 4H), 5.66 (t, J = 5 Hz, 1H), 5.15 (s, 2H), 3.78 (m, 2H), 3.40-3.51 (m, 20H), 3.17 (q, J = 6 Hz, 2H), 2.94 (br s, 6H), 2.89 (s, 2H), 2.39 (s, 3H), 2.32 (s, 2H), 1.88 (m, 2H), 1.77 (p, J = 6 Hz, 2H), 1.01 (s, 6H), 0.25 (s, 9H).



Iodide **9** (65 mg, 77 μmol) was dissolved in ethanol (2 mL) and treated with Hunig's base (2 drops) followed by FITC (33 mg, 84 μmol). After 30 m, TLC (4/0.9/0.1 : CH_2Cl_2/MeOH/NH_4OH) showed product but a little starting amine remaining so more FITC (12 mg) was added. After 1 h, the reaction mixture was concentrated onto silica gel (2 g) and chromatographed (silica gel, 0 to 20% MeOH in CH_2Cl_2) to give **12** (84 mg, 88%) as a yellow glass. LC/MS shows a major peak with m/z = 1236.9 $[M+1]^{+}$and 619.2 $[M + 2]^{2+}$. ^{1}H-NMR (DMSO-d6) δ 7.96 (s, 1H), 7.77 (d, J = 7 Hz, 1H), 7.69 (s, 1H), 7.65 (d, J = 7 Hz, 1H), 7.33 (d, J = 7 Hz, 1H), 7.18 (t, J = 7 Hz, 1H), 7.18 (d, J = 8 Hz, 1H), 7.16 (d, J = 7 Hz, 1H), 6.76 (d, J = 2 Hz, 1H), 6.72 (dd, J = 2, 8 Hz), 6.67 (br s, 2 H), 6.57 (br s, 4H), 5.66 (br t, J = 5 Hz, 1H), 5.15 (s, 2H), 3.74 (br m, 2H), 3.41-3.50 (m 20 H), 3.16 (m, 2H), 2.94 (br s, 6H), 2.89 (s, 2H), 2.39 (s, 3H), 2.32 (s, 2H), 1.88 (p, J = 6 Hz, 2H), 1.77 (p, J = 6 Hz, 2H), 1.01 (s, 6H).



Iodide **5** (62 mg, 51 μmol), hexamethylditin (22 mg, 14 μL, 67 μmol) and tetrakis triphenylphosphine palladium(0) (2 mg., 1.5 μmol) were slurried in dioxane (1 mL), purged with nitrogen for 30 m and heated to 100 ºC for an hour. The reaction was concentrated, dissolved in DMSO and chromatographed (50 g Isco C18, 0 to 100% MeOH with 0.2% formic acid in each) to give the tin compound **5** (29 mg., <50%) as an orange solid. This compound was identical to prepared previously using an alternate route (**3** to **4**).

(1) Hughes, P. F., Barrott, J. J., Carlson, D. A., Loiselle, D. R., Speer, B. L., Bodoor, K., Rund, L. A., and Haystead, T. A. J. (2012) A highly selective Hsp90 affinity chromatography resin with a cleavable linker. *Bioorganic & Medicinal Chemistry 20*, 3298-3305.